A report is offered of a double blind study, placebo controlled, which compared production lots of Edmonston B and further attenuated strains of live measles virus vaccine. The trial was made in Tegucigalpa, Honduras. Results are presented and discussed.

EDMONSTON B AND A FURTHER ATTENUATED MEASLES VACCINE—A PLACEBO CONTROLLED DOUBLE BLIND COMPARISON

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Introduction

IN 1962, Schwarz[1] reported preliminary studies of a live measles virus vaccine strain that appeared to cause less fever than the attenuated Edmonston B strain. Krugman, et al.,[2] comparing the two strains, observed that febrile responses exceeding 103° F which were induced by the vaccines occurred approximately half as frequently following Schwarz's strain vaccine as following the Edmonston B strain vaccine.

In 1963, a scientific group from the World Health Organization[3] recommended field studies in different countries to compare several further attenuated strains, among them the Schwarz strain, with the attenuated Edmonston B strain. Studies in progress have been summarized by Cockburn and Pecenka,[4] and some results from Yugoslavia,[5] Nigeria,[6,7] Russia,[8] and Canada,[9] are already available.

In previous trials, subjects were vaccinated with experimental batches of vaccines. More recently, the possibility has been raised that some further attenuation of each of the strains may have been achieved in the course of large-scale commercial production. The purpose of the present study was to evaluate clinical and serologic responses following injection with measles vaccine from recently produced, commercial lots of vaccine. Unmodified Edmonston B and Schwarz (hereafter called "further attenuated") strains of live measles vaccines were employed in a placebo controlled, double blind field trial in Honduras.

Material and Methods

Setting

The field trial took place in Tegucigalpa, the capital of Honduras, during February and March, 1965. A single neighborhood, Barrio Morazon (population 4,100), was selected because of its compact size and proximity to a health center, which was its principal medical facility. No house in the area was more than a ten-minute walk from the health center.

Study Population

Parental consent was obtained for 300 children between the ages of four months and four years, and all susceptible to measles according to their histories, to participate in the study. The children were matched for age, and then they were randomly divided into three equal

groups. One group was to receive Edmonston B strain vaccine; the second, further attenuated strain vaccine; and the third, a placebo.

A physician examined each candidate for signs of illness. All children with fever, marasmus, known tuberculosis, or allergy to eggs were rejected. Each child's height and weight were measured.

Vaccines and Vaccination

Single lots of Edmonston B vaccine* and of further attenuated vaccine† were used.‡

The two vaccines and the placebo were lyophilized preparations in single-dose vials. The materials were reconstituted immediately before use. A 0.5 cc dose of vaccine or placebo was injected by syringe and needle subcutaneously in the left deltoid region.

All injection materials including diluent were disguised with colored labels. The nurse in charge of vaccination did not participate in surveillance of reactions.

* "Rubeovax," Lot #74814B, was kindly provided by Maurice R. Hilleman, Ph.D., of the Merck Institute for Therapeutic Research.

† "Lirugen," Lot #185016B, was kindly supplied by Anton J. F. Schwarz, M.D., of the Pitman-Moore Company.

‡ Names of commercial manufacturers and trade names are provided for identification only, and inclusion does not imply endorsement by the Public Health Service of the US Department of Health, Education, and Welfare.

Surveillance

Ten nurse auxiliaries were assigned to districts with from 25 to 30 children each. Each district contained children in all three study groups. The auxiliaries who performed surveillance did not know to which vaccine group a child had been assigned.

The homes of all children were visited daily between 2:00 P.M. and 6:00 P.M. for three weeks after vaccination. The visiting auxiliary recorded rectal temperatures and other symptoms on a standard reaction form. One of three physicians visited each child every second or third day and saw every child with elevated temperature or rash. If a child developed a temperature of 39° C or greater, he was given pediatric aspirin.

Serology

A serum sample was collected from each child before vaccination, and more than 95 per cent of the children returned in 25 days for a postvaccinal bleeding. A microadaptation of the measles hemagglutination inhibition (HI) test of Rosen,[10] using African green monkey erythrocytes, was used to test pre- and postvaccinal sera simultaneously. As soon as the second blood sample had been taken, the placebo group were vaccinated, but they were not subsequently followed for clinical or serologic response.

Table 1—Honduras measles vaccine trial, 1965, final study population

	Number of children in each vaccine group			
	Edmonston B	Further attenuated	Placebo	Total
Initial registration	94	91	93	278
Less seropositive*	9	13	17	39
Less inadequate information†	2	7	5	14
Final study population	83	71	71	225

* HI titer equal or greater than 1:5 before start of study.
† Children observed fewer than six times and children missing one of paired sera.

Table 2—Honduras measles vaccine trial, 1965, distribution of maximum temperature* day 4 through 14 after vaccination

Vaccine	Number of children in each temperature interval					Total
	<38°	38°–38.9	39°–39.4	39.5–39.9	40°+	
Edmonston B	8 (9.9%)	28 (34.6%)	21 (25.9%)	15 (18.5%)	9 (11.1%)	81†
Further attenuated	19 (27.9%)	29 (42.6%)	10 (14.7%)	7 (10.3%)	3 (4.4%)	68‡
Placebo	39 (54.9%)	29 (40.8%)	1 (1.4%)	1 (1.4%)	1 (1.4%)	71

* Rectal temperatures expressed in degrees Centigrade.
† Excluding two children who did not demonstrate seroconversion (HI titer of 1:5 or greater following vaccination).
‡ Excluding three children who did not demonstrate seroconversion (HI titer of 1:5 or greater following vaccination).

Results

Study Population

Table 1 shows the distribution by vaccine group of the final study population. Of the 300 children asked to participate in the study, 10 never appeared at the vaccination center and 12 were rejected because of a contraindication to measles vaccination; 278 subjects were registered and vaccinated. A total of 53 vaccinees were excluded from the study: 39 demonstrated an HI measles antibody titer of 1:5 or greater before vaccination; and for 14, information was inadequate either because of missing or insufficient serologic specimens or too few home visits. Every child in the study was seen at least six times during the 11 days from day 4 through day 14 after vaccination; 90 per cent of the entire study population were seen at least ten times during this 11-day observation period.

There were no significant differences between the three groups in age, average height, and weight.

Clinical Response

A rectal temperature of 39.5° C (103.1° F) or higher was observed approximately twice as frequently following vaccination with Edmonston B strain vaccine as following vaccination with further attenuated vaccine; 29.6 per cent of those vaccinated with Edmonston B and 14.7 per cent of those vaccinated with further attenuated vaccine registered one or more readings of 39.5° C from day 4 through day 14 following vaccination (Table 2). This difference between the two vaccine groups is statistically significant ($p=0.05$).

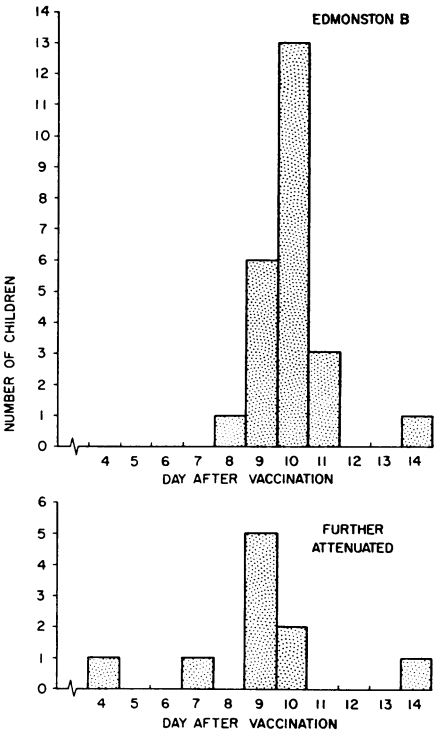
Children one year old and older developed fever of 39.5° C and higher following measles vaccination more frequently than infants (Table 3). However, this difference in febrile response

Table 3—Honduras measles vaccine trial, 1965, maximum temperature ≥39.5° C in three age groups, day 4 through 14 after vaccination

Vaccine	Number of children in each age group							
	<12 months		12–23 months		24+ months		All ages	
	Total	≥39.5° C	Total	≥39.5° C	Total	≥39.5° C	Total	≥39.5° C
Edmonston B	17	3 (17.6%)	34	13 (38.2%)	30	8 (26.7%)	81*	24 (29.6%)
Further attenuated	15	1 (6.7%)	28	4 (14.3%)	25	5 (20.0%)	68†	10 (14.7%)
Placebo	15	0 (0.0%)	29	1 (3.4%)	27	1 (3.7%)	71	2 (2.8%)

* Excluding two children who did not demonstrate seroconversion.
† Excluding three children who did not demonstrate seroconversion.

Figure 1—Honduras measles vaccine trial day of maximum temperature, day 4 through 14, only children with temperature ≥39.5° C



between infants and older children was not statistically significant.

The group receiving Edmonston B strain experienced most of its high fevers on day 10 following vaccination, while the group receiving further attenuated strain developed most of its febrile reactions on day 9. This is depicted graphically in Figure 1.

Reactions other than fever are compared in Table 4. One recipient of Edmonston B vaccine, a 13-month-old male, experienced a single generalized seizure ten days after vaccination. His temperature was 39.8° C at the time of the convulsion. He was observed daily for eight days following the seizure, and no further neurologic abnormalities were noted.

A transient rash was found in over one-fourth of the children receiving Edmonston B strain. Approximately 12 per cent of subjects receiving further attenuated strain and 10 per cent of those receiving placebo developed rash. Cough and diarrhea were common in the community, and there was no difference in the incidence of these symptoms. There was no difference in the frequency of conjunctivitis between the placebo and either of the vaccinated groups. Tonsillitis consisting of redness, edema, and rarely exudate, was more frequently observed in the vaccinated groups than in the placebo group.

Serologic Response

Over 95 per cent of the children receiving vaccine developed antibodies by the 25th day after vaccination. None of those receiving placebo developed measles HI antibodies. The geometric mean HI titer (Table 5) was significantly higher following vaccination with Edmonston B strain than following vaccination with the further attenuated strain ($p<0.001$).

Table 4—Honduras measles vaccine trial, 1965, reactions following vaccination, day 4 through 14 after vaccination

Vaccine group	Total	Number of children with each symptom for one day or more				
		Rash	Cough	Diarrhea	Conjunctivitis	Tonsillitis
Edmonston B	81*	22 (27.2%)	56 (69.1%)	45 (55.6%)	15 (18.5%)	25 (30.9%)
Further attenuated	68†	8 (11.8%)	47 (69.1%)	40 (58.8%)	4 (5.9%)	16 (23.5%)
Placebo	71	7 (9.9%)	49 (69.0%)	31 (43.7%)	10 (14.1%)	2 (2.8%)

* Excluding two children who did not demonstrate seroconversion.
† Excluding three children who did not demonstrate seroconversion.

Table 5—Honduras measles vaccine trial, 1965, serologic response 25 days after vaccination

Vaccine	No. paired sera	No. converted*	% Seroconversion*	Geometric mean HI titer of seroconvertors*			
				<12 mos.	12–23 mos.	24+ mos.	All ages
Edmonston B	83	81	97.6	97.8	135.7	96.5	111.6
Further attenuated	71	68	95.8	66.4	80.0	64.1	70.6
Placebo	71	0	0.0	NA	NA	NA	NA

* As indicated by appearance of HI titer of 1:5 or greater, 25 days after vaccination.
NA=Not applicable.

Five children did not develop HI antibodies detectable at a level of 1:5 or greater after vaccination. Three of these, five, six, and seven months old, were vaccinated with the further attenuated vaccine. Two of the five, seven months and two years old respectively, received Edmonston B vaccine. One two-year-old child in the Edmonston B group developed a titer of 1:5 only. The distribution of all postvaccinal measles HI titers is shown in Figure 2.

All infants eight months old and older developed HI antibodies after vaccination, as did ten of fourteen infants (71 per cent) four to seven months old. Table 6 provides the serologic response of infants by their age in months.

Discussion

Measles is a severe and frequently fatal disease in much of the world, particularly the developing nations. Hence there is ample justification for trials in these areas to evaluate the safest, most practical, and most effective means of immunization. Clearly, regimens involving multiple visits to the vaccination center or concomitant injections of gamma globulin have the disadvantage of increased demand for trained personnel and expensive materials.

This double blind controlled evaluation of two currently available commercial lots of single-dose live measles virus vaccines indicates that the further attenuated vaccine causes high fever less frequently than the Edmonston B vaccine. However, the systemic symptoms associated with vaccine-induced illness were mild for both vaccines. As was noted early in the experience with live

Figure 2—Honduras measles vaccine trial serologic response 25 days after vaccination

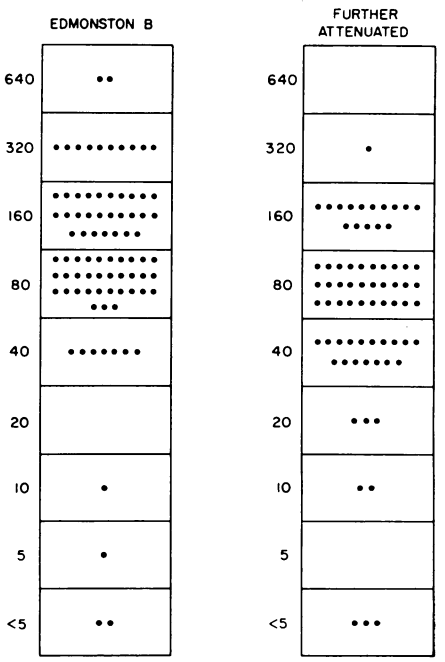


Table 6—Honduras measles vaccine trial, 1965, serologic response of infants 25 days after vaccination

	Edmonston B									Further attenuated								
	Age in months								All infants	Age in months								All infants
	4	5	6	7	8	9	10	11		4	5	6	7	8	9	10	11	
Susceptible*	0	1	1	3	1	4	3	5	18	1	2	4	2	2	5	1	1	18
Seroconverted†	0	1	1	2	1	4	3	5	17	1	1	3	1	2	5	1	1	15

* HI titer $\leq$1:5 at start of study.
† HI titer $\geq$1:5 25 days after vaccination.

measles virus vaccine,[11] high fever was infrequently accompanied by toxicity. In Chile[12] and Upper Volta,[13] after small-scale field trials, it was concluded that reactions caused by the unmodified Edmonston B vaccine did not warrant the inclusion of gamma globulin in the measles vaccination regimen. Successful mass campaigns have been conducted in both countries with the Edmonston B strain alone.[14,15]

Other studies in which the Edmonston B and the further attenuated Schwarz strain vaccines have been compared are summarized in Table 7. The results of the present study most resemble those of Krugman, et al.[2] However, many variables, such as study design, age, and nutritional status of the study population, season, type of reaction surveillance, and methods of analysis, operate in this type of immunization trial. Although results are not strictly comparable from study to study, it is most doubtful that the two recently produced commercial lots of vaccine tested in this trial differ significantly in their reactogenicity from their respective experimental lots tested previously.

Both vaccines produced an excellent immunologic response. Only five susceptible children, four of whom were in-

Table 7—Comparative studies of live measles vaccines, febrile reaction rates*

		Vaccine strain tested		
Investigators	Country	Edmonston B	Edmonston B + gamma globulin	Schwarz
Krugman, et al.[2]	United States	30.0	15.0	15.0
Morley, et al.[6]	Nigeria†	NT	8.8	4.6
Nagler, et al.[9]	Canada	59.2	27.8	16.2
Milovanovic[5]	Yugoslavia	39.4	37.0	NT
Hendrickse, et al.[7]	Nigeria‡	NT	8.3	12.8
Present study	Honduras	29.6	NT	14.7

* Per cent of susceptible children with temperature $\geq$103° F.
† Imesi-Ilesha.
‡ Eruwa.
NT=Not tested.

fants, failed to develop detectable HI antibodies. Presumably in these infants unmeasurable passively acquired maternal antibody prevented vaccine virus multiplication.[16]

The group vaccinated with Edmonston B vaccine developed a significantly higher geometric mean HI titer than did the group who received the further attenuated strain. Krugman[17] has also described this difference in titer which becomes more accentuated two years after vaccination. Krugman has suggested, on the basis of the absence of clinical measles in vaccinated children under the New York Health Insurance Plan, that, regardless of the height of antibody titer, both vaccines offer effective long-term prophylaxis against challenge with natural measles.

The measles mortality rate, both in the United States[18] and in West Africa,[19] is highest for children from 6 to 24 months old. Thus, the question of the earliest age at which protection can be offered is of practical importance. In this study, all infants eight months old or older produced HI antibodies in response to vaccination. In fact, a high percentage (71 per cent) of infants four to seven months old responded serologically to vaccination. Furthermore, infants as a group had fewer high fevers than the older children in the study.

These results suggest, as first implied by data of Stokes, et al.,[20] that the optimal age for immunization with unmodified live measles vaccines is between 8 and 12 months. In situations where the measles mortality rate for young infants is high, it may be desirable to vaccinate earlier, for example at six months, even though some vaccinees may not be protected.

Conclusion

A placebo controlled, double blind study compared production lots of the Edmonston B and further attenuated strains of live measles virus vaccine. A neighborhood of Tegucigalpa, Honduras, was the locus of the trial. Daily reaction surveillance was conducted by a team of auxiliary nurses and physicians. About 30 per cent of susceptible children vaccinated with Edmonston B vaccine and about 15 per cent of those vaccinated with further attenuated vaccine exhibited fever of 39.5° C (103.1° F) or greater, from 4 to 14 days after vaccination. Over 95 per cent of both vaccine groups developed measles HI antibodies by the twenty-fifth day after vaccination.

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REFERENCES

1. Schwarz, A. J. F. Preliminary Tests of a Highly Attenuated Measles Vaccine. Am. J. Dis. Child. 103:386–389, 1962.
2. Krugman, S.; Giles, J. P.; Jacobs, A. M.; and Friedman, H. Studies with a Further Attenuated Live Measles-Virus Vaccine. Pediatrics 31:919, 1963.
3. WHO Hlth. Org. Tech. Rep. Ser. No. 263, Measles Vaccines. Geneva, Switzerland: World Health Organization, 1963.
4. Cockburn, W. C., and Pecenka, J. Measles Vaccine Studies Sponsored by the World Health Organization —1964. Arch. Gesamte Virus-forschung XVI:257, 1965.
5. Milovanovic, M. V. Live Measles Vaccine Trials in Yugoslavia. Ibid. XVI:231, 1965.
6. Morley, D. C.; Woodland, M.; Krugman, S.; Friedman, H.; and Grab, B. Measles and Measles Vaccination in an African Village. Bull. World Health Organ. 30:733–739, 1964.
7. Hendrickse, R. G.; Montefiore, D.; Sherman, P. M.; and Van der Wall, H. M. Studies on Measles Vaccination in Nigerian Children. Brit. M. J. 1:470–474, 1964.
8. Smorodintsev, A. A.; Boychuk, L. M.; Shikina, E. S.; Meshalova, V. N.; Taros, L. Y.; Aminova, M. G.; Revenok, N. D.; and Safarov, D. I. Prevention of Measles by Use of Live Vaccines in the USSR. Arch. Gesamte Virus-forschung XVI:285–293, 1965.
9. Nagler, F. P.; Foley, A. R.; Furesz, J.; and Martineau, G. Studies on Attenuated Measles-Virus Vaccines in Canada, 1964. Bull. World Health Organ. 32:791–801, 1965.
10. Rosen, L. Hemagglutination and Hemagglutination-

Inhibition with Measles Virus. Virology 13:139, 1961.
11. Katz, S. L.; Kempe, C. H.; Black, F. L.; Lepow, M. L.; Krugman, S.; Haggerty, R. J.; and Enders, J. F. Studies on Attenuated Measles-Virus Vaccine. VIII. General Summary and Evaluation of the Results of Vaccination. New England J. Med. 263:180–184, 1960.
12. Ristori, C.; Boccardo, H.; Miranda, M.; and Borgono, M. A Controlled Trial of Live-Virus Vaccine Against Measles in Chile. Bull. World Health Organ. 30:763–768, 1964.
13. Meyer, H. M., Jr.; Hostetler, D. D., Jr.; Bernheim, B. C.; Rogers, N. G.; Lambin, P.; Chassary, A.; and Smadel, J. E. Response of Volta Children to Live Attenuated Measles Virus Vaccine. Ibid. 30: 769–781, 1964.
14. Ristori, C.: Boccardo, H.; Borgono, J. M.; and Miranda, M. Mass Vaccination Against Measles in Chile with the Enders Live Vaccine. Arch. Gesamte Virus-forschung XVI:246–250, 1965.
15. Meyer, H. M., Jr. Mass Vaccination Against Measles in Upper Volta. Ibid. XVI:243–245, 1965.
16. Krugman, S.; Giles, J. P.; Friedman, H.; and Stone, S. Studies on Immunity to Measles. J. Pediat. 66:471–488, 1965.
17. Krugman, S. Measles Vaccine. Letter to the editor. New England J. Med. 273:563, 1965.
18. Langmuir, A. D. Medical Importance of Measles. Am. J. Dis. Child. 103:224–226, 1962.
19. Cantrelle, P. Mortalité et morbidité par Rougeole dans les Pays Francophones de l'Ouest Africain. Arch. Gesamte Virus-forschung XVI:35, 1965.
20. Stokes, J., Jr.; Reilly, C. M.; Hilleman, M. R.; and Buynak, E. B. Use of Attenuated Measles Virus Vaccine in Early Infancy. New England J. Med. 263: 230–233, 1960.

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